Chapter

The Role of Renin Angiotensin Aldosterone System in the Pathogenesis and Pathophysiology of COVID-19

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Abstract

The novel coronavirus also known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) whose origin is still having uncertainties related to the existence of an intermediate host, has created the currently ongoing pandemic of coronavirus disease 2019. (COVID-19) The binding assays of SARS-CoV-2 spike protein receptor binding domain disclosed enhanced affinity with human angiotensin II-converting enzyme receptor (hACE2) comparing to the bat ACE2 receptors. ACE2, is an essential component of the regulatory mechanism of the renin-angiotensin-aldosterone system, (RAAS) and this pathway is considered to interact with the pathophysiology of COVID-19. In this chapter, we will discuss the key role of RAAS in the pathogenesis of SARS-CoV-2.

Keywords: ACE2, RAAS, SARS-CoV-2, COVID-19, Ang II, ADAM17

1. Introduction

1.1 The pathogenic interaction of SARS-CoV-2 and renin: angiotensin: aldosterone system

Coronaviruses (CoVs) belong to the family of Coronaviridae which is further divided into four genera as Alphacoronavirus, (α-CoV) Betacoronavirus, (β-CoV) Gammacoronavirus, (γ-CoV) and Deltacoronavirus. (δ-CoV) [1] α- and β-CoVs are able to infect mammals, while γ-and δ-CoVs tend to infect birds [1]. HCoV-229E, HCoV-NL63 (α-CoVs) and HCoV-OC43, HCoV-HKU1 (β-CoVs) have crossed the species barriers from their bat reservoirs via various intermediate hosts to humans, and caused mild endemic infections of the upper respiratory tract such as common colds [2]. However, in recent years, several epidemic β-CoVs which were associated with severe acute respiratory syndrome (SARS) such as SARS-CoV-1, and middle east respiratory syndrome (MERS) such as MERS-CoV were considered as potential emergent pathogens for global pandemics [3, 4]. Most recently novel coronavirus (NCoV-19) also known as SARS-CoV-2 (β-CoV) which shows 96% genomic similarity with bat SARS-like coronavirus strain, BatCov RaTG13 have created the currently ongoing pandemic of coronavirus disease 2019. (COVID-19) [5, 6].

SARS-CoV-2 has a round or elliptic shape, often pleomorphic with a diameter of approximately 60–140 nm, and a nucleocapsid core surrounded by a lipid bilayer

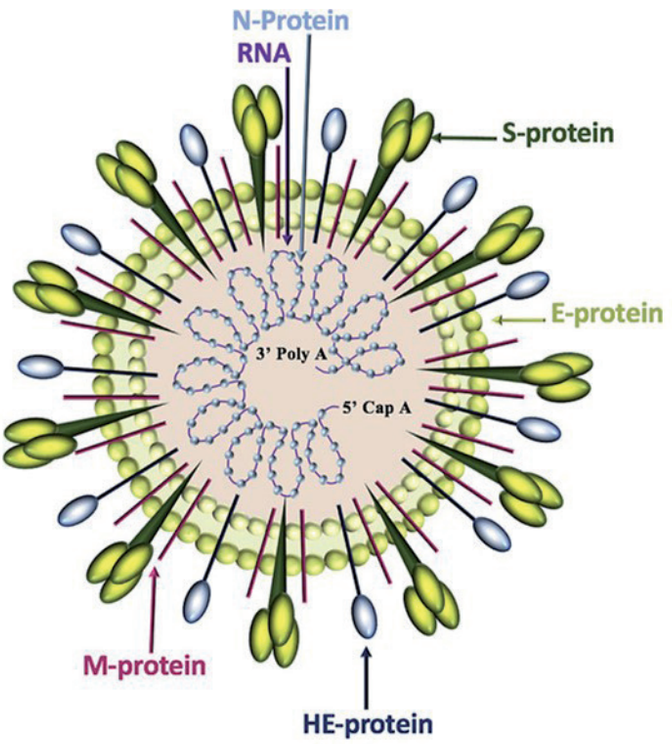

Figure 1.
Coronavirus structure. (Adapted from Fehr et al. [7]).

envelope (**Figure 1**) [7]. The nucleocapsid core contains the viral genome, single-stranded, non-segmented, positive-sense RNA which has a 5′ cap and a 3′ poly-A tail with a length of ~26.4 to ~31.7 kilobase (kb) complexed with the structural nucleocapsid (N) proteins (**Figure 1**) [7]. The lipid bilayer envelope which is taken by budding of RNA/nucleocapsid complex into the lumen of the ERGIC (endoplasmic reticulum (ER)–Golgi intermediate compartment) has the other structural glycoproteins including the spike (S) protein, the membrane (M) protein, the envelope (E) protein, and a fifth protein called hemagglutinin-esterase (HE) protein which binds to the terminal sialic acid residues on the host cell membrane glycoproteins and it manifests acetyl-esterase activity for the egress of SARS CoV-2 (**Figure 1**) [7, 8]. S protein, ~180 kDa glycoprotein is initially cleaved by the host serine protease furin resulting non-covalently linked transmembrane S2 subunit and a protruding extracellular S1 subunit during the intracellular maturation in the trans-Golgi-network [9]. Plasma membrane-exposed or secreted furin also cleaves S protein during entry of the virus resulting S1/S2 protomers which appear as mushroom-like trimers on the viral envelope (**Figure 1**) [9]. Each of the protomers can have an open or closed conformation. The "open" conformation of S1 exposes the receptor binding domain, (RBD) containing receptor binding motif (RBM) which shows increased binding affinity with angiotensin II-converting enzyme (ACE2) [10]. Currently circulating SARS-CoV-2 variant has a S1 D614G mutation with N-linked glycosylation sites N165 and N234 which favor the open conformation resulting the SARS-CoV-2 D614G variant more infectious [11]. SARS-CoV2 is also unique for having a proline residue between S1 and S2 subunits which leads to the formation of a turn/stem-loop structure resulting O-linked glycosylation at the cleavage site residues S686, S673, and T678 [12]. Following the binding of the amino- (N) terminal of S1 subunit RBM to ACE2, transmembrane protease serine 2 (TMPRSS2) and furin-mediated proteolytic activation/cleavage between the carboxyl- (C) terminal of S1 and N-terminal of S2 subunits results conformational change and fusion of the virus envelope and host cell membrane via C- terminal of S2 subunit, and this process delivers the virus genome into the host cell (**Figure 2**) [8]. SARS-CoV-2 also

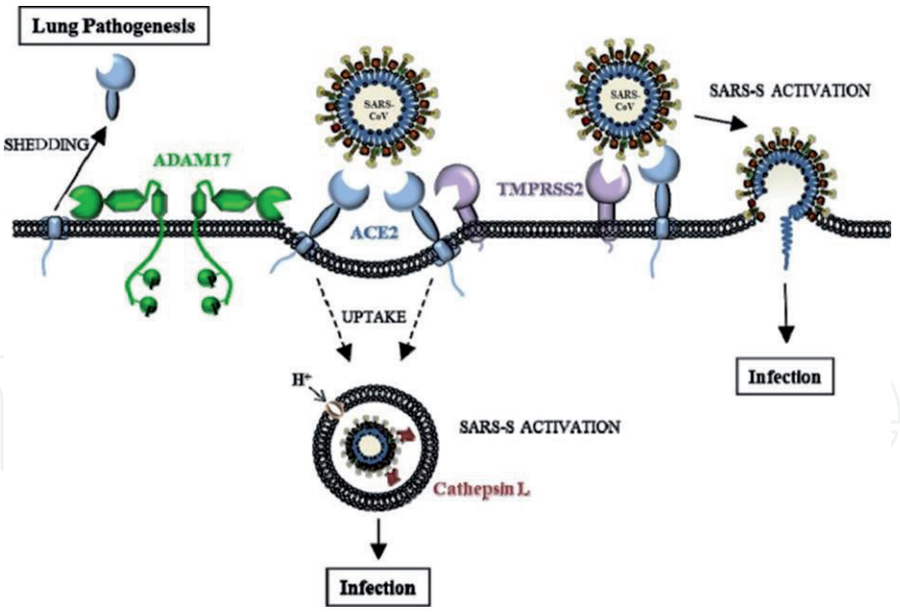

Figure 2.
Role of host cell proteases in the cellular entry of SARS-CoV. (Adapted from Heurich et al. [8]).

enters the host cell via receptor-mediated endocytosis (**Figure 2**) [8]. Upon binding of N terminal of S1 subunit RBM to ACE2 virion is taken up into the endosome, where S1 and S2 subunits are cleaved and activated by the pH-dependent cysteine protease, cathepsin L (**Figure 2**) [8]. Conformational change of the S1and S2 subunits allows fusion of the virus envelope with the endosomal membrane and release of the viral genome into the cytoplasm (**Figure 2**) [8]. ADAM17 (a disintegrin and metalloprotease 17) also known as tumor necrosis factor-α (TNF- α) converting enzyme, (TACE) is a membrane protease involved in the endogenous shedding of ACE2 complexed with S1 subunit RBD from the cell membranes (**Figure 2**) [8]. ADAM17-dependent ACE2 shedding is believed to promote lung pathogenesis [8, 13]. ACE2, is an essential component of the regulatory mechanism of the renin-angiotensin-aldosterone system (RAAS), and this pathway is considered to interact with the pathophysiology of COVID-19. In this chapter, we will discuss the key role of RAAS in the pathogenesis of Covid-19.

2. The renin: angiotensin: aldosterone system and pathophysiology of Covid 19

The RAAS is an important hormonal homeostatic mechanism of the body that involves the liver, kidneys, lungs and adrenal glands which plays a critical role in the regulation of blood pressure, fluid/electrolyte balance, systemic and pulmonary vascular resistance and vascular remodeling [14]. The function of the RAAS is mainly regulated by angiotensinogen, prorenin, renin, angiotensin I, (Ang I) angiotensin II, (Ang II) aldosterone, angiotensin 1–7, (Ang 1–7) angiotensin 1–9, (Ang 1–9) angiotensin I-converting enzyme, (ACE) and ACE2 (**Figure 3**) [15]. Prorenin, the precursor of renin, is proteolytically activated in the kidney by neuroendocrine convertase 1 (proprotein convertase 1) or cathepsin B, and nonproteolytically in many tissues by the renin/prorenin receptors [14]. Renin is produced by the juxtaglomerular cells in response to sympathetic nervous system (SNS) stimulation, hypotension, decreased cardiac output (CO) and renal perfusion pressure, decreased distal tubular sodium and chloride concentration and dehydration (**Figure 3**) [15]. Angiotensinogen, synthesized in the liver is an α-2-globulin, a member of the serpin family of proteins, but unlike the other serpins it is not known to inhibit proteases (**Figure 3**) [14, 15].

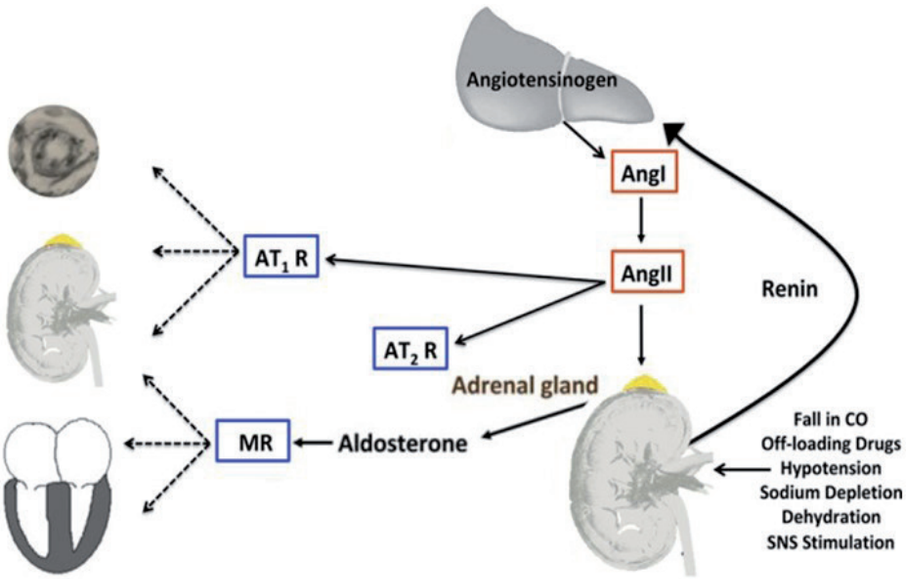

Figure 3.
The renin-angiotensin-aldosterone system (Adapted from Ames et al. [15]).

It has an elongated N-terminus as a substrate for renin which cleaves 10 N-terminus amino acids from angiotensinogen and creates the decapeptide Ang I (**Figure 3**) [15]. Ang I is considered to have no direct biological activity other than being a precursor to Ang II which is synthesized by ACE through removal of two C-terminal residues from Ang I (**Figure 3**) [15, 16]. ACE belongs to the M2 gluzincin family of metalloproteinases, zinc-dependent peptidyldipeptidase and it exists in two forms, somatic ACE and testicular ACE [16]. Both are derived from the same gene, controlled by alternative promoters [17]. Testicular ACE is considered to play a role in male fertility and sperm physiology [18]. Somatic ACE (ACE) is expressed in high amounts by the vascular endothelium of the lungs, renal proximal tubular epithelium and ciliated intestinal epithelium [17]. ACE mRNA expression has also been identified in different cell types and tissues including macrophages, dendritic cells, (DC) choroidal plexus and brain [19, 20]. The ACE gene promoter has been shown to harbor CpG islands which regulate ACE gene expression during inflammation via TNF-α, dependent hypermethylation resulting a decrease in cellular ACE activity [21, 22]. ACE is an integral membrane protein, which can be also cleaved by ACE secretases to produce a circulating form of the enzyme [23]. This soluble ACE activity is shown to be inhibited by an endogenous inhibitor which restricts ACE mediated Ang I conversion in the systemic circulation irrespective to the concentration of the circulating ACE that confines Ang II mediated responses in the tissues [24]. Changes in ACE expression have been shown to have minimum effect on blood pressure due to renin-mediated compensation of Ang I and its bioactive endogenous byproduct angiotensin 1–12 (Ang 1–12) which is more specialized for controlling blood pressure than Ang II [25, 26]. Ang II is considered to be mostly associated with innate and adaptive immunity, oxidative stress, inflammation and fibrosis [27, 28].

The Ang II receptors, (ATR1) and (ATR2), are a class of G protein-coupled receptors sharing a sequence identity of ~30%, but having a similar affinity for Ang II, which is their main ligand (**Figure 3**) [15, 16]. ATR2 stimulates the G protein-coupled receptor Gi subunit, and primarily inhibits the cAMP-dependent pathway by inhibiting adenylyl cyclase activity and decreasing the production of cAMP from ATP, which in turn results decreased activities of the cAMP-dependent protein kinases [29]. The downstream signaling pathways of these inhibitory processes lead to the modulation of protein kinase A (PKA), protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) pathways, eventually inhibiting inflammation and

growth-specific functional processes mostly affecting cardiac and vascular tissues [30]. ATR2 activation was reported to induce the activation of peroxisome proliferator-activated receptor, (PPARγ) a powerful anti-inflammatory factor in the post-ischemic cardiac tissue of rabbits that was accompanied by a down-regulation of MAPKs p42/44 [31]. ATR1 which stimulates the G protein-coupled receptor Gq protein alpha subunit on the vascular smooth muscle cell membranes which in turn activates an inositol triphosphate (IP3)-dependent mechanism leading to increase intracellular calcium levels and vasoconstriction (**Figure 3**) [15, 27]. Ang II stimulates aldosterone secretion from the adrenal gland cortex (**Figure 3**) [15]. Aldosterone increases sodium, chloride and bicarbonate reabsorption coupled with potassium and hydrogen excretion from the distal convoluted tubules, and amplifies the pathophysiologic effects of Ang II in the heart, kidney and vasculature via acting on the mineralocorticoid (MR) receptors (**Figure 3**) [15, 24]. More importantly, aldosterone was associated with inflammation via ER unfolded protein responses, mitochondrial dysfunction, as well as increased synthesis of pro-inflammatory cytokines such as interleukin 6. (IL-6) [32, 33]. It was also disclosed that activation of ATR1-receptors promotes Ang II-induced reactive oxygen species (ROS) generation, inflammation and angiogenesis via stimulating the Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, (NOX) nuclear factor kappa-light-chain-enhancer of activated B cells, (NF-κB) extracellular signal-regulated kinases, (ERK1/2) MAPK and signal transducer and activator of transcription 1 (STAT1) pathways [34, 35]. NOX is the best known non-mitochondrial source of ROS generation [36]. p22phox subunit of NOX is required for activating, stabilizing and/or regulating NOX homologs [36]. Ang II reportedly induces oxidative stress by elevating the expression of p22phox [37]. ROS are considered to oxidize membrane phospholipids, proteins and nucleic acids, and lead to tissue hypertrophy and inflammation mainly in the alveolar epithelial cells, endothelium and heart via triggering the synthesis of adhesion molecules including intercellular adhesion molecule 1, (ICAM-1) vascular cell adhesion protein 1, (VCAM-1) monocyte chemoattractant protein 1, (MCP-1) and macrophage colony stimulating factor. (M-CSF) [34, 35]. Moreover, Ang II stimulates production of ROS from NO leading to depletion of NO causing further injury to blood vessels [14]. Additionally, C- reactive protein (CRP) induces ATR1 transcription and translation as well as enhanced ATR1 levels in blood vessel wall [14, 35]. Heat shock proteins (HSPs) have been found to be a regulator of NF-κB cascade in inflammation induced by Ang II via activation of the inhibitor of nuclear factor kappa B (IκB) kinase (IKK) complex and phosphorylation of IκBα. This process leads to ubiquitination and degradation of IκBα, and permits NF-κB translocation to the nucleus. NF-κB stimulates the transcription of proinflammatory cytokines including TNF-α, IL-6, IL-8, MCP-1, and cyclooxygenase [38]. Cyclooxygenase 1-derived prostaglandin E2 and prostaglandin E2 type 1 receptors are considered to play a role in Ang II-dependent hypertension via AT1R/phospholipase A2 pathway which promotes ROS production coupled with Ca2+ influx [39]. TNF-α, primary substrate for ADAM17 is cleaved and released from the cell membrane forming the soluble TNF-α which in turn binds and activates TNF-α receptors on the cell surfaces [40]. ADAM17 activity is upregulated by the binding of soluble TNF-α to its receptors, and also via the ATR1/Ang II axis [40]. Ang II enhances activation of MAPK cascades including ERK1/2, c-Jun N-terminal kinase (JNK) and ERK5 via ATR1 resulting increased synthesis of matrix metalloproteinase-2 (MMP-2) which amplifies the inflammation associated with the proinflammatory cytokines and results to angiogenesis, widespread disruption of endothelial barriers and cardiac abnormalities [41]. Activation of STAT1/STAT2 downstream pathway via Ang II – ATR1 binding stimulates interferon-stimulated genes (ISG) expression by inducing the interferon-stimulated response element

(ISRE) promoter and increases the maturation and activation of the antigen presenting cells, natural killer cells and T-box expressed in T cells (T-bet) cells which are bridging between innate and adaptive immunity and leading to autoimmune reactions and cardiovascular diseases [42]. Ang II-induced vasoconstriction and inflammatory endothelial cell injury have been associated with accelerated thrombus development in the arteries, veins, and capillaries via activation of different components of the coagulation cascade [42, 43]. ACE2 is a zinc-carboxypeptidase consisting of 805 amino acids with an extracellular N-terminal domain, transmembrane (TM) domain and an intracellular C-terminal tail (**Figure 4**) [44]. The zinc-binding motif (HEMGH) is located within the carboxypeptidase domain which also recognizes RBM of S1 subunit of SARS-CoV-2 (**Figure 4**) [44]. Collectrin domain is the site for ACE2 shedding with ADAM17 and TMPPSS2, and it is crucial for interacting with neutral amino acid transporters (**Figure 4**) [44]. ACE2, which has 42% identical nucleotide sequence with ACE indicating that the two genes, ACE2 and ACE arise through duplication [45]. ACE2 is expressed in a diverse group of cells including the oral, nasal, type II lung alveolar, tongue and esophageal epithelial cells, enterocytes, endothelial cells, cardiomyocytes, arterial smooth muscle cells in most organs, cortical neurons and glia, renal tubules, ductal cells, bladder urothelial cells and male reproductive cells [44–46]. ACE2 cleaves the carboxyl (C)-terminal amino acid phenylalanine from Ang II, (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) and hydrolyses it into the vasodilator Ang (1–7), (Asp-Arg-Val-Tyr-Ile-His-Pro) a ligand for the G-protein coupled receptor Mas receptor (MasR) (**Figure 5**) [47]. ACE2 also converts Ang I to Ang 1–9 that can be further hydrolyzed to Ang 1–7 by the action of neprilysin, (Nep) which is a zinc-dependent metalloprotease, and cleaves peptides at the N-side of hydrophobic residues (**Figure 5**) [47, 48]. Nep also directly converts Ang I to Ang 1–7 which is further enzymatically decarboxylated to alamandine, a ligand for Mas-related G-protein-coupled receptor, member D (MrgD) (**Figure 5**) [47, 49]. Activation of Mas and MrgD receptors upon binding with Ang 1–7 and alamandine respectively promote anti-inflammatory responses via increasing the levels of anti-inflammatory cytokines IL-4 and IL-10 which have been disclosed in macrophages and microglial cells [50, 51]. ACE2 is considered as a key modulator of the RAAS via regulating physiological and pathological functions of cardiovascular, renal and pulmonary systems via counterbalancing the hypertensive, vasoconstrictor, hypertrophic and inflammatory effects of ACE [47, 52]. Thus, the ratio of Ang II/ACE2 plays an important role in the pathogenesis of several diseases including Covid-19 [47, 52, 53]. Two forms of ACE2 have been reported [44].

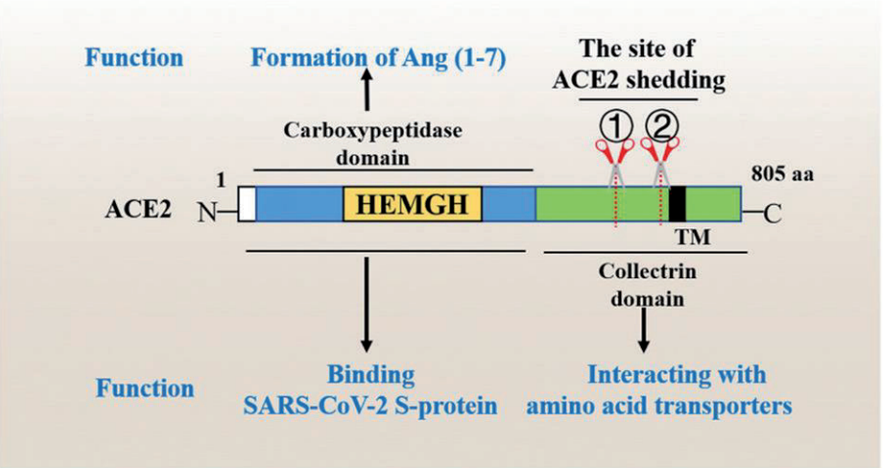

Figure 4.
Domain structure and function of angiotensin-converting enzyme 2. (Adapted from Bian et al. [44]).

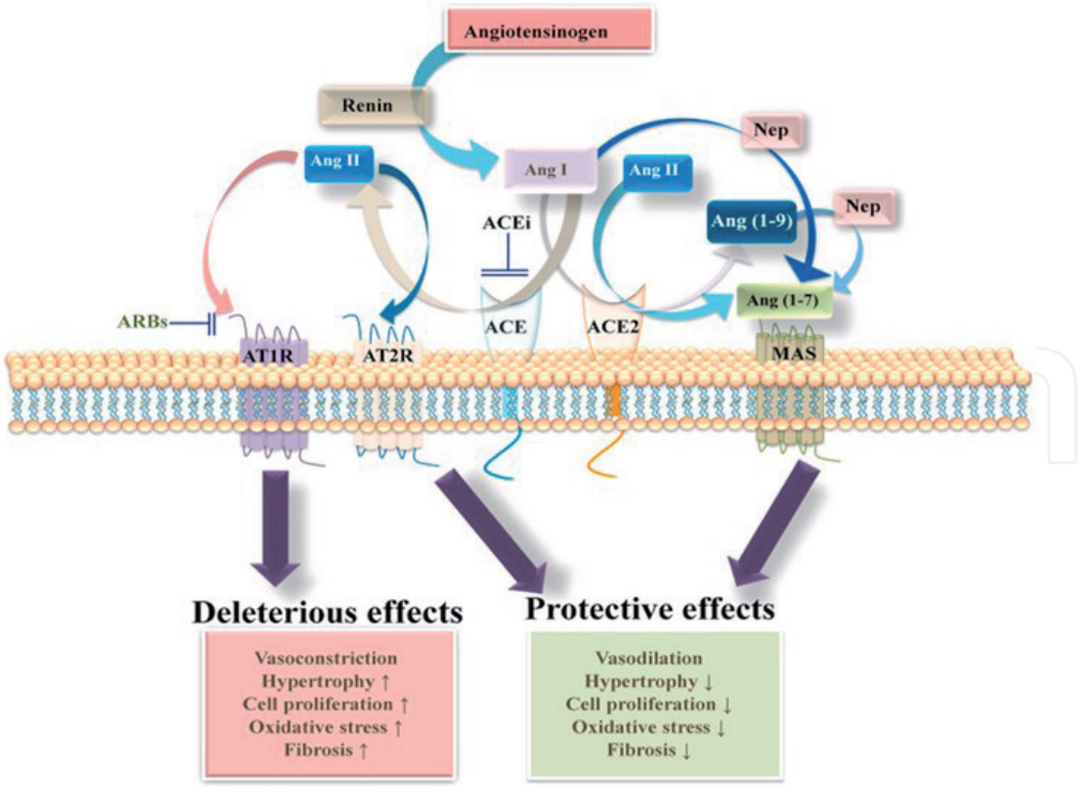

Figure 5.
A classic model of RAAS showing deleterious and protective effects. (Adapted from Gul et al. [47]).

The full-length membrane-bound ACE2 (mACE2) is located on the apical surface of epithelial cells, differently from ACE, which is located between the apical and basolateral membranes in polarized cells [19, 44]. S1 trimeric subunit RBM of SARS-CoV-2 binds to the widely expressed mACE2 extracellular domain (**Figures 2** and **4**) [8, 44]. The second form soluble ACE2 (sACE2) is shed into the circulation via Ang II/AT1R/ADAM17 axis (**Figure 2**) [8, 54, 55]. Although the levels of sACE2 may be increased in plasma or urine in some pathological processes, such as hypertension, the expression levels of mACE2 is not affected [45]. The majority of ACE2 is membrane-bound, and it has a compensatory balancing effect on the RAAS [49]. Ang II counterbalances the number of mACE2 via cellular internalization through endocytosis and degradation in the lysosomes, thus inhibits the antioxidative, anti-inflammatory, anti-hypertrophic and antifibrotic effects of ACE2 [55]. ACE2 inactivity via shedding, cellular internalization and degradation at early stages of COVID-19 might have a disturbing effect on the RAAS homeostasis which leads to increased vascular permeability, fluid accumulation in the extra-alveolar spaces, oxidant/antioxidant imbalance and impaired tissue repair [56, 57]. COVID-19 patients were found to suffer more frequently from severe endothelial injury due to the membrane damage by binding of SARS-CoV-2 [58]. Widespread thrombosis with microangiopathies were reported, and the COVID-19 patients were found 9 times more likely to experience alveolar capillary microthrombi, and 2.7 times more likely to experience intussusceptive angiogenesis than the patients with flu [59]. This phenomenon of thrombosis and other vascular events in COVID-19 were considered more likely associated with abundance of ACE2 on the endothelial cell membranes which permit SARS-CoV-2 infection along the endothelium resulting endothelial damage, complement activation, release of Von-Willebrand factor from the endothelial cells, hypercoagulability and microthrombi formation [58, 59]. ACE2 expression is regulated by genetic and epigenetic factors, body mass index, inflammatory cytokines, cigarette smoking, sex hormones and aging. ACE2 expression in different

tissues across human individuals were found to be high in Asian ethnic groups [60]. The upregulation of ACE2 expression was associated with the decline in the levels of estrogen and androgen, aging, inflammation and cigarette smoking [60, 61].

Cis-elements in the proximal promoters of ACE2 genes have binding sites for canonical interferon- (IFN) dependent transcription factors including ISRE/STAT1, interferon regulatory factor 1 (IRF1), IRF3/7 and IRF8 [62]. Type I IFNs, and to a lesser extent type II and type III IFNs have been shown to upregulate ACE2 expression especially in the human upper airway basal cells and bronchial cells [63]. Higher enrichment of ISRE/STAT1/3 and/or IRF3/7 binding sites were detected in single cell RNA-sequence data sets from the nasal epithelium and upper airway goblet secretory cells of the patients with the severe manifestations of COVID-19 suggesting dual roles of ACE2 in the pathogenesis of SARS- COV-2 [64]. First, ACE2 serves as an innate immune receptor for SARS-CoV-2 which might compete with Ang II for the binding sites located at the carboxypeptidase domain of ACE2, and SARS-COV-2 gains access into the cells via ACE2 receptor. The similar functional innate protein–protein interactions between the human toll-like receptors (TLRs) TLR1, TLR4, and TLR6 with a binding energy values of −57.3, −120.2, −68.4 respectively, being the TLR4-S protein interaction strongest have been demonstrated by the molecular docking [65]. Secondly, innate IFN responses against SARS-CoV-2 upregulate ACE2 expression on the cell membranes which augments anti-inflammatory responses via counter-balancing the effects of Ang II, but also allows further cellular entry of SARS-CoV-2. However; the balancing arm of the RAAS functioning as ADAM17 mediated ACE2 shedding and ADAM17 mediated TNF-α activation/hypermethylation of the CpG islands at the ACE gene promoter eventually decrease cellular entry of SARS-CoV-2 and ACE expression.

In summary, there are couple of balancing and counter-balancing factors existing in the pathogenesis of Covid-19. The main counter-balancing arm is between Renin/ACE/Ang II/ATR1 and ATR2/ACE2/Mas/MrgD. The second counter-balancing arm is between the innate immune responses including SARS- CoV-2 induced IFN response/increased membrane expression of ACE2, and ADAM17 mediated ACE2 shedding activated by ATR1/Ang II and TNF-α mediated downregulation of ACE expression. The second arm has its own feedback via TNF-α. However, the main arm counter-balancing the protective and deleterious effects of RAAS which is being abused by SARS- CoV-2 via competing with Ang II for binding to the ACE2 receptor plays a crucial role in the pathogenesis of Covid-19 via the unopposed effects of Ang II including oxidative stress, inflammation, stimulation of innate and adaptive immunity via T-bet cells, thrombosis, angiogenesis and fibrosis.

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